THE DISTRIBUTION OF ELECTROLYTES IN PHASCOLOSOMA MUSCLE

H. BURR STEINBACH

(From the Department of Zoölogy, Columbia University, and the Marine Biological Laboratory, Woods Hole, Mass.)

In recent years considerable attention has been paid to the distribution of electrolytes in tissues, particularly muscle and nerve. It has been recognized, of course, that the total electrolyte content of such a tissue as muscle must be distributed between at least two phases, one being roughly the cellular phase, the other extracellular.

That protoplasm is a solution of electrolytes, among other things, has been amply demonstrated by observations on conductivity. Gross analysis of masses of cells and tissues shows that these electrolytes must be largely of a type with inorganic cations and organic anions (see Fenn, 1936). Many of the organic substances in cells have been described, but until recently little attention has been paid to the distribution of the inorganic constituents.

The general principle that, in many tissues composed of highly differentiated cells, chloride does not penetrate into the cells (Fenn, *loc. cit.* See also Amberson et al, 1938) is very useful, since it allows calculations to be made of the approximate amounts of other inorganic constituents present in the extracellular space and hence, cellular concentrations, if the total concentration in the tissue is known. The method of study is very simple, involving soaking the tissues in chosen solutions and then analyzing them for chloride and the other elements in question. The data then are treated on the assumptions that all electrolytes of the external medium are in simple equilibrium with the extracellular space (chloride space) and that the total amounts of metallic elements found in the ash may be regarded as a source of positively charged ions, either within or without the cells.

A recent study (Steinbach, 1940) dealing with chloride, sodium, potassium and calcium in *Thyone* muscle has been reported. The results given in the present paper are concerned with similar experiments on another invertebrate smooth muscle, the retractor muscle of the marine annelid *Phascolosoma*.

The structure of these muscles has been described by Olson (1940).

Methods

The methods used were identical with those reported previously (Steinbach, 1940). The muscles were excised with as little injury as possible and immersed in fifty to one hundred times their weight of solution. Solutions used were either normal sea water, isotonic salt solutions, isotonic buffered sucrose solutions or mixtures of these as indicated. All solutions had a pH of 8.0 to 8.5. The experiments were carried out at room temperature.

Results

Phascolosoma muscles swell when transferred to sea water and their composition changes accordingly. Table I gives average figures for

Table I

Analysis of *Phascolosoma* muscle, fresh (column 1) and treated with sea water for several hours (column 2). Column 3 gives the analysis of *Phascolosoma* body fluid and column 4 of Woods Hole sea water.* Figures in brackets give number of analyses averaged. Concentrations in milliequivalents per hundred grams.

Element	(1) Fresh	(2) Soaked	(3) Body fluid	(4) Sea water
Na	12.2 [3]	14.9 [6]	37.8 [2]	44
K	10.6 [4]	9.0 [2]	3.8 [2]	0.9
Ca	0.85 [4]	1.14 [4]	2.1 [3]	1.8
Cl	9.1 [8]	16.0 [9]	43.0 [2]	51
Relative weight	100	119		
Dry weight %	22		9	
Ash weight %	2.1			

* Sea water is not constant in composition from year to year. The figure for chloride, for example, is from data of 1938. In 1939 the chloride was 2 to 3 per cent lower.

Na, Cl, K and Ca contents of muscles freshly removed from the body and muscles that have been soaked in sea water for several hours. Figures are also included in the table showing the concentrations of these elements in body fluid of *Phascolosoma* and in Woods Hole sea water.

The change in chloride of the muscles can be almost entirely accounted for by assuming that the change of weight represents an increase in the extracellular space. Sodium is present in fresh muscle slightly in excess of chloride. In soaked muscles the situation is changed and most of the chloride can be accounted for as sodium

chloride. A little potassium is lost on soaking and calcium increases slightly.

The main purpose of this work was to follow the loss or gain of cellular electrolytes, using the chloride space (ratio of Cl inside to Cl outside) as a measure of the extracellular space of the whole tissue. In order to do this, it was first essential to show that chloride in the tissue bore a simple linear relationship to the chloride concentration of the medium. Muscles were soaked in various dilutions of sea water with isotonic sucrose solutions and then analyzed. A few experiments

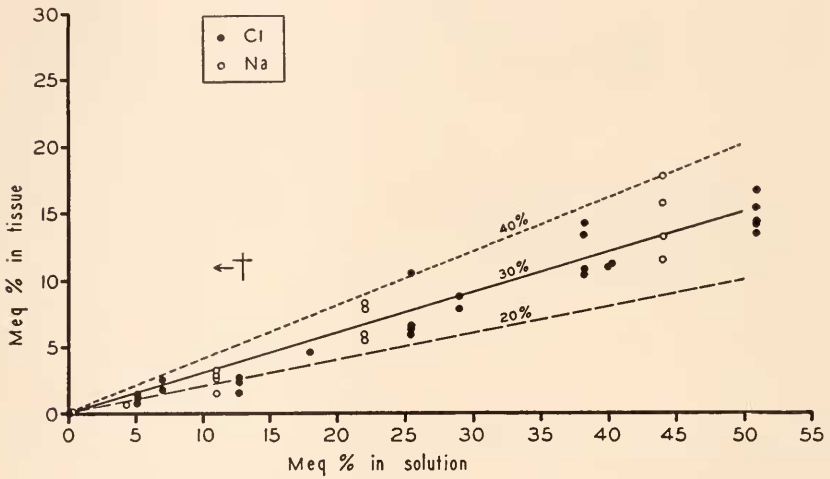

FIG. 1. Chloride (●) and sodium (○) concentrations in *Phascolosoma* muscles plotted against the same elements in solution. The concentrations in the solutions were altered by diluting normal sea water with isotonic buffered sucrose solutions. Tissues soaked 2 to 4 hours before analysis. The cross indicates the minimal salt concentration for maintaining irritability (response to electrical stimulation). All concentrations in milliequivalents per hundred grams solution or final wet weight of tissue. Straight lines represent calculated values for chloride space as indicated. Each point is a single determination on several muscles.

were also done in which normal sea water was diluted with an artificial sea water (Allen's formula) made up with nitrates instead of chlorides. The results (Fig. 1) show clearly that chloride in the muscle does bear a simple linear relationship to chloride of the medium and hence the assumption of an extracellular chloride space is probably correct. On this basis, about 30 per cent of the whole tissue is extracellular space. Determinations of sodium were made on muscles similarly treated and since sodium appears to be extracellular in soaked muscles the results are included in Fig. 1.

The behavior of potassium in the muscles was studied by immersing muscles in solutions of varying potassium content made by diluting either an artificial potassium-free sea water (Allen's formula) or normal sea water with isotonic KCl solution. For each solution being tested, two batches of muscles were used, the one to be analyzed for

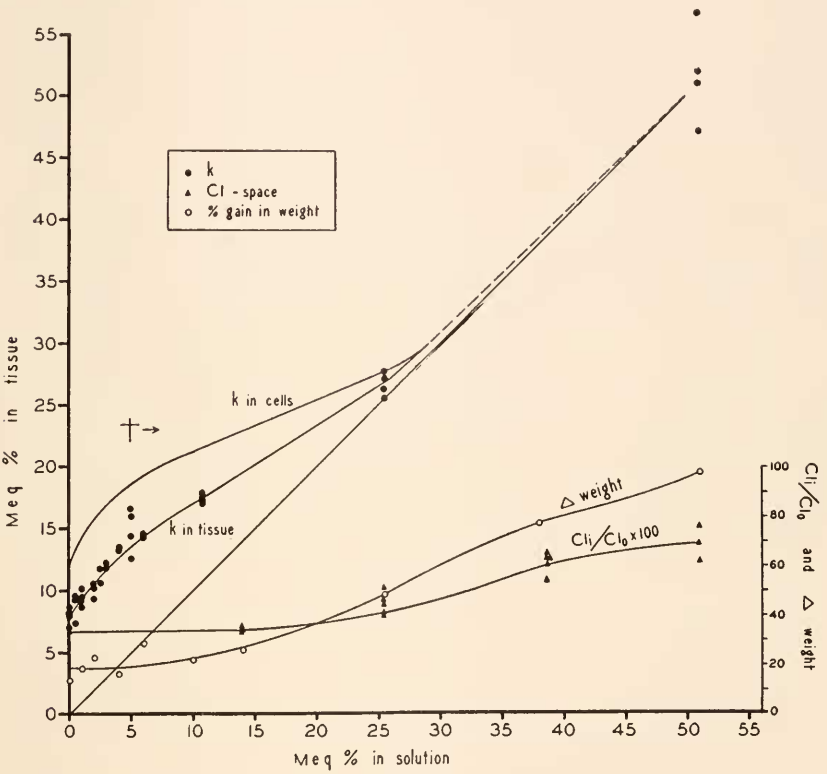

Fig. 2. Potassium concentrations in *Phascolosoma* muscles (●), weight changes (○) and chloride space (▲) plotted against potassium concentrations in the external medium. Tissues soaked 6 to 8 hours. Solutions made up by diluting sea water with potassium chloride solutions. Cross indicates highest potassium concentration at which irritability is maintained. The curve for potassium in the cells represents potassium concentrations calculated as outlined in Table II. Concentrations in milliequivalents per hundred grams solution or final wet weight of tissue.

potassium, the other for chloride. Initial and final weights were recorded. The results are reported in terms of milliequivalents per hundred grams (meq per cent) final wet weight of tissue. Figure 2 summarizes the results. Potassium is lost from the tissue to K-free

solutions and is gained in increasing amounts as the potassium content of the medium is increased. Potassium is first concentrated in the tissue but the amount gained for each increment in potassium of the medium becomes less with higher concentrations until, when the solution is about 0.25 M in potassium, tissue and solution have nearly equal concentrations. Further increases in potassium of the medium do not appear to disturb this last relationship. The potassium concentration in the cells was estimated by subtracting the potassium present in the chloride space ($Cl_i/Cl_o \times$ K-conc. of solution) from the total potassium and dividing the results by the cell space ($1 - Cl_i/Cl_o$). Values

TABLE II

Calculated figures for potassium concentrations in the cells (column 5) and cell volume (column 6). Calculations performed using figures read from the smoothed-out curves drawn through the experimental points of Fig. 2. Concentrations in milliequivalents per hundred grams.

Relative weight (column 2) $= \frac{\text{final weight}}{\text{initial weight}}$. $Cl_i/Cl_0 = \frac{\text{chloride in tissue}}{\text{chloride in solution}}$.

K concentration in cells (column 5) calculated by subtracting product $Cl_i/Cl_0 \times K$ in solution from K concentration of tissue and dividing by cell space $(1 - Cl_i/Cl_0)$. Cell volume calculated by multiplying relative weight by cell space. Average chloride of all solutions assumed to be 52 meq per cent.

(1) Conc. K solution (fresh muscle)	(2) Relative weight tissue	(3) Conc. K tissue	(4) Cl_i/Cl_0	(5) Conc. K cells	(6) Cell volume
	100	10	0.17	11.8	83
0	119	8	0.33	11.9	80
5	120	14	0.33	18.5	80
10	123	17	0.33	22.0	82
15	128	20	0.35	22.8	83
20	133	23	0.37	24.7	84
25	139	26	0.42	26.7	81
35	171	35	0.57	34.8	73
50	199	50	0.67	50.0	66

for these calculations were read from the smoothed-out curves of Fig. 2 and the results are plotted in Fig. 2 (upper curve). These results merely indicate that the concentration of cellular potassium is considerably higher than the potassium concentration of the medium at first, the difference decreasing with increasing concentrations until when the solution is about 0.25 M in potassium the two phases are equally concentrated. Since the cells contain most of the dry substance, this means that, on the basis of water content, the cells always contain a higher concentration of potassium than is found in the external medium.

The calculations of cellular potassium concentrations are calculations based on the assumption that chloride does not penetrate the living cells. That is, cellular potassium concentration figures really represent the amount of potassium present in excess of chloride and presumably occupying space not penetrated by chloride. The mechanism for partitioning the elements in this fashion may or may not be the cell membrane but whatever it is, it has the interesting result that "cell space" remains almost constant in spite of the fact that the muscle swells to almost double its original size. This fact is brought out clearly by the figures given in Table II. These figures are calculated from data read from the smoothed-out curves of Fig. 2. Cell size (volume of muscle not containing chloride) is calculated by multiplying the final relative weight (initial weight = 100) by the cell space ($1 - Cl_i/Cl_o$). From the figures shown it is apparent that these muscles can increase in weight by as much as 40 per cent and more than double the chloride space without any appreciable change in the calculated cell volume. It would seem rather doubtful that this actually means a constant cell size. A more liberal and probably more correct interpretation of the results would be that the protoplasm, due to its whole chemical and physical structure, represents non-solvent space as far as chloride is concerned. Permeability might be a factor in this but modified Donnan equilibria or other chemical equilibria must also be important. This interpretation probably holds also for *Thyone* muscles but is not needed so obviously since those tissues do not swell to any great extent in solutions of high potassium concentration.

The calcium balance in the tissue was studied by methods similar to those used for potassium, except that dilutions were made with calcium chloride solutions. The results of the studies are shown in Fig. 3. There is a nearly linear relationship between the calcium of the muscle and calcium of the solutions except that a small residue remains in the muscle even after prolonged soaking in calcium-free solutions. The slope of the line drawn through the experimental points is greater than that of Fig. 1, showing that some calcium is probably entering the cells. On the other hand, the results also show that calcium is never as concentrated in the cells as in the external medium so long as the outside concentration is above that of normal sea water.

As the calcium of the medium is increased, swelling of the tissues becomes less and the chloride space increases. This may indicate that excess calcium actually causes a shrinkage of the cells but it might also show that some cells are being destroyed. Calculations show that not only is total cellular calcium lower in tissues in high-calcium solutions,

but the concentration of calcium in the cells is also lower. This is similar to the situation found in *Thyone* (Steinbach, 1940) and shows that there must be some change in the cells under the influence of high external calcium so that less calcium can enter or stick to them.

Calculations similar to those made for potassium show that as the calcium of the medium increases the cell size decreases. This is ap-

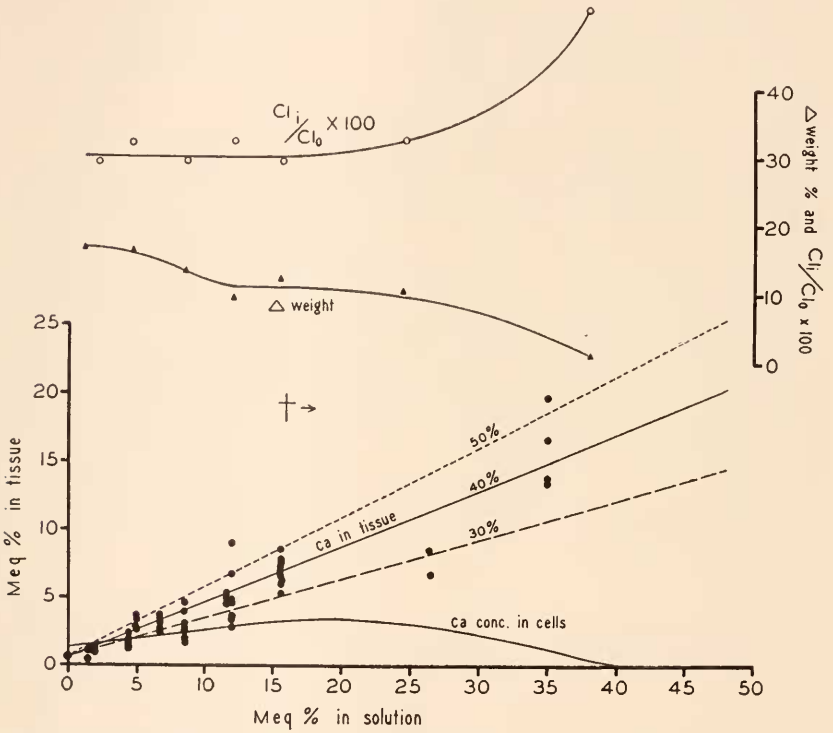

FIG. 3. Changes in *Phascolosoma* muscles as the calcium concentration of the medium is altered. Symbols, concentrations and methods of calculation as in Fig. 2.

parent from inspection of Fig. 3. The total weight of the tissue is less when the calcium is high outside and the chloride space increases; therefore there must be a decrease in cell (non-chloride) space. This may mean either shrinkage or destruction of cells. These results do not differentiate between the two possible changes.

Discussion

The concept of the chloride space of muscle tissue is a useful one. The evidence is good that under normal conditions all of the chloride is extracellular in frog striated muscle, and is quite satisfactory for other vertebrate tissues as well (see Manery and Hastings, 1938). Normal *Thyone* muscle appears to contain chloride only in the extracellular spaces and the present evidence would show that the same holds true in *Phascolosoma*. Both these latter muscles would be classed as smooth muscles; thus another similarity is shown between smooth and striated muscle tissue.

The constancy of the chloride space under normal conditions and the regular way in which it changes with various experimental procedures can be regarded as evidence that there is some definite exclusion of chloride from part of the muscle volume. It is, however, a little difficult to conclude that the cells of *Phascolosoma* muscle change as little in size as is shown in Table II, when the whole muscle doubles in weight. It seems more probable that the partition of chloride is not a definite exclusion by a discrete membrane but depends upon a number of chemical and physical factors so balanced that there is a certain volume of tissue that is, for want of a better term, non-solvent space with respect to chloride. This non-solvent space for chloride probably is nearly equivalent to cell space in normal tissue. But in greatly swollen tissue this may or may not be true. Some start has been made toward a histological study of muscles swollen in KCl and the results, while inconclusive, would indicate that the cells do not stay the same size under all these conditions. It probably is best, then, to relate chloride in swollen muscles to the organic content of the protoplasm rather than to size of cells. On this basis the calculated cell sizes given in Table II would then show that the organic (dry weight) constituents of the cells were still present, occupying space and influencing chemical equilibria as they do in normal cells.

On this basis, the concentration of potassium in such a manner that there is always more potassium inside cells than outside, figured per unit weight of water, probably represents potassium entering into some special equilibrium with the protoplasmic constituents rather than being locked within a membrane. The two mechanisms, of course, have the same effect, but a membrane explaining the results reported here would have to possess most remarkable properties.

Several problems arise in connection with the potassium studies. Potassium enters the muscle cells so that there is always more potassium

inside than outside. There can be little sodium within the cells and other inorganic cations are present in low concentration. Since chloride does not enter, there is then the problem of accounting for the mode of existence of the potassium. The cells are obviously in osmotic equilibrium with their normal environment and yet the internal potassium content can be doubled without a change in calculated cell size. Potassium then enters where there was no potassium before, no swelling occurs and there is no common cation exchanged or anion entering. The only possible solution would seem to be the creation of new electrolytes, probably organic acids, from non-electrolytes previously active only in maintaining osmotic balance. There is at present no clue as to the nature of this process or how it might take place.

The calcium balance of the cells presents as much of a problem as the potassium balance but here the mechanisms concerned allow a partial penetration of calcium, not a concentration of the element. Except with very low external calcium, there is always less of the element in the tissue than in the medium. Furthermore, the changes in calcium content of the cells indicate that the ability to take up calcium from the environment is lowered by treatment with excess calcium in the medium. In view of the demonstrated importance of calcium in surface phenomena (see Heilbrunn, 1937), it seems probable that calcium never penetrates completely within the cell but is stopped by some surface equilibrium condition.

Summary

An analysis is presented of the major inorganic constituents of *Phascolosoma* muscle and body fluid. As is usually found in muscle tissue, potassium is more concentrated in the tissue than in the body fluid or in sea water. Calcium is in about the same concentration while sodium and chloride are in the tissue in lower concentrations than they exist in the external medium.

Sodium and chloride appear to be extracellular in these muscles, most of the potassium and some calcium are intracellular. Parallel analyses of muscles for different elements show that the cells can take up considerably more potassium than they normally contain without an increase in chloride. Some calcium can also be taken up by the cells.

These findings are discussed briefly in connection with their bearing on problems of permeability to electrolytes of muscle cells.

LITERATURE CITED

AMBERSON, W. R., T. P. NASH, A. G. MULDER AND DOROTHY BINNS, 1938. The relationship between tissue chloride and plasma chloride. *Am. Jour. Physiol.*, **122**: 224.

FENN, W. O., 1936. Electrolytes in muscle. *Physiol. Rev.*, **16**: 450.

HEILBRUNN, L. V., 1937. An Outline of General Physiology. Saunders and Co. Philadelphia.

MANERY, JEANNE F., AND A. B. HASTINGS, 1939. The distribution of electrolytes in mammalian tissues. *Jour. Biol. Chem.*, **127**: 657.

OLSON, M., 1940. *Biol. Bull.*, **78**: 24.

STEINBACH, H. B., 1940. Electrolytes in Thyone muscles. *Jour. Cell. and Comp. Physiol.* **15**: 1.